tion. Lateral flow based RDT's typically incorporate a sample pad material designed to capture red blood cells ("RBCs") without promoting hemolysis, as the RBCs and hemoglobin can interfere with the colorimetric read of the device. The present system has no such requirement; that is, 6% whole blood in buffer may be added directly to the device with no separation of cellular components prior to running the standard assay protocol described above.

[0176] Representative whole blood assay results are provided in FIGS. **35**-**37**, which present whole blood performance in direct comparison to plasma from the same blood tube. FIG. **37** shows comparison of whole blood and plasma performance on the exemplary system. Cartridges were processed with 6% whole blood or 3% plasma from the same venipuncture sample approximately 24 hours after draw. The larger volume of whole blood was to approximately compensate for cell volume. The array layout was as in FIG. **28**. Images for the whole blood and plasma from this HIV-positive sample were essentially identical (as shown in FIGS. **35** and **36**), demonstrating that there is no cellular or hemoglobin-induced interference in the system. The bar graph provides quantitative output for the gp41, p24, and anti-human IgG sites on the arrays, demonstrating the same quantitative signal profile for the whole blood and plasma samples. This particular sample had strong reactivity to the gp41 antigen but very little reactivity to p24.

[0177] Note that the anti-human IgG is printed at very low concentration in the array, with activity tuned to give signal on the same scale as positive antigen sites. Total IgG is in large excess in the sample. Data presented are mean values for three replicate cartridges for each sample type. Error bars represent one standard deviation.

[0178] Reproducibility. The whole blood and plasma assay described above and shown in FIG. **6** include triplicate measurements of the same blood or plasma sample (6 cartridges total), providing an initial assessment of system reproducibility. Defining % coefficient of variation ("% CV") as the standard deviation of the three measurements divided by the mean, the following results were observed. For the plasma samples, gp41 S/CO % CV was 10%. For the whole blood samples it was 13%. For the anti-human IgG sites, the plasma samples gave 11% CV and the whole blood gave 4%. These are reasonable initial results for a 28 minute whole blood assay; CVs are expected to improve as manufacturing controls are augmented during scale-up.

Example 9

Simple Fluorescence Sandwich Assay

[0179] FIG. **38** shows a series of diagrams representing the steps of an exemplary, indirect fluorescence assay. The goal of this type of assay is to detect and identify the presence of specific antibodies in a biological sample. In one configuration, one or more antigens ("Ag") are immobilized on an assay surface, such as the walls of a microwell device (e.g., a 96-well microtiter plate). The antigen can be, for example, a purified natural protein, recombinant protein, synthetic peptide, or other biological molecular representing an immunogenic target. For commercially prepared immunoassays, this antigen immobilization step is performed during manufacture of the immunoassay kit. At the time of use, a biological sample is introduced to the well (i.e., Step "1)" shown in FIG. **38**). The biological sample may potentially include target and non-target antibodies ("Ab"). If present, target antibodies bind to the immobilized antigens during an incubation period. A wash step is typically used to remove excess biological sample (i.e., step "2)" shown in FIG. **38**), then a labeled detect antibody is added to the well (i.e., Step "3)" shown in FIG. **38**). For example, in an assay for specific human antibodies, the detect antibody could be a goat anti-human IgG conjugated with horseradish peroxidase ("HRP"). The label may be an enzyme (HRP, alkaline phosphatase, luciferase, etc.), for use with colorimetric or chemiluminescence signal transduction. The label could also be a fluorescent dye, lanthanide, nanoparticle, microparticle, light scattering particle, or some other labeling mechanism. A final wash step is typically used to remove excess labeled detect antibody (i.e., step "4)" shown in FIG. **38**), and the microwell device is then read by eye (e.g., in a colorimetric assay) or through some appropriate instrumentation (e.g., absorbance plate reader, chemiluminescence detector, or fluorescence detector).

[0180] In another configuration (not shown), antigens may be printed to a solid substrate that is then incorporated in a fluidic cartridge that includes a fluid inlet port. The antigens may be printed, for example, in a spot or stripe, and multiple antigens may be printed in an array format. Similarly to the microwell protocol described above, a biological sample is added to the fluidic cartridge and, if present, target antibodies bind to the printed antigens. After a wash step to remove excess biological sample, a labeling step, then another wash step to remove excess labeled detect antibody, the fluidic cartridge may be imaged using a reader instrument (see, for example, Myatt, C. J. et al., Low-cost, multiplexed biosensor for disease diagnosis, Proc. SPIE 7167 Frontiers in Pathogen Detection: From Nanosensors to Systems, 716703 (2009), which is incorporated herein in its entirety).

[0181] While the indirect fluorescence assay has proven utility, the multi-step process may be undesirable for certain applications, particularly in the context of rapid, point-of-care or point-of-need testing. A potentially simpler workflow approach is known in the field as the "double antigen sandwich" method (See, for example, U.S. Pat. No. 6,120,990 to Brust et al. and U.S. Pat. No. 7,629,295 to Wienhues et al.). The double antigen sandwich method takes advantage of the multi-valent nature of immunoglobulin ("Ig") molecules. Instead of the anti-immunoglobulin detect format used in the indirect fluorescence assay described above, the detect reagent is a labeled antigen containing the same binding epitope as the immobilized antigen. The antigen may be, for example, a purified natural protein, recombinant protein, synthetic peptide, or other biological molecular representing an immunogenic target. The label may be an enzyme (e.g., HRP or alkaline phosphatase), for use with colorimetric or chemiluminescence signal transduction. The label may also be a fluorescent dye, nanoparticle, microparticle, light scattering particle, or some other labeling system. In the double antigen configuration, the target antibody is sandwiched between the surface immobilized antigen and the labeled detect antigen.

[0182] There may be several advantages to the double antigen sandwich approach. Importantly, the format provides detection of multiple immunoglobulin types (IgG and its subtypes, IgM, etc.). The format also is amenable to simplified workflow protocols, as wash and detect reagent steps may be eliminated.

[0183] Various versions of the double-antigen sandwich concept have been described in the literature and incorporated into commercially-available assays, including, for example, tests for detection of anti-HIV antibodies such as SD Bioline